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14. ABSTRACT

The purpose of the studies conducted in the first reporting period was to begin to investigate the effect of pharmacologicstimulation of A2A or A2B adenosine receptors on trauma/hemorrhagic shock-induced organ injury in rats. The A2A receptoragonist 2-p-(2-carboxyethyl)phenethyl-amino-5'-N-ethyl-carboxamidoadenosine CGS21680 (0.1 and 0.5 mg/kg)exacerbated organ injury when mixed into Ringer's Lactate resuscitation fluid, which was associated with a long-lastinghypotensive effect of this agent. In contrast, the A2B receptor agonist BAY 60-6583 (0.5 mg/kg) mixed into Ringer'sLactate resuscitation fluid almost completely prevented trauma/hemorrhagic shock-induced lung injury while having noeffect on the blood pressure of rats. We conclude that A2B receptor stimulation is a promising approach in curbinghemorrhage-induced organ failure in the battlefield.

15. SUBJECT TERMS
hemorrhage, shock, resuscitation, lung injury, inflammation, neutrophil, endothelial cell, epithelial cell, gut

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Introduction

The major cause of death in potentially salvageable battlefield casualties is hemorrhage. Consequently, strategies directed at preventing the direct (shock) and secondary adverse consequences (organ failure) of hemorrhage would offer the greatest opportunity for reducing mortality and morbidity. The direct consequences of shock are countered by resuscitation with crystalloid solutions, such as Ringer's lactate (RL). RL, however, does not prevent the organ failure that is secondary to hemorrhage, and it is the injury of end-organs, such as lung, gut, and kidney that leads to death following battlefield injuries. Organ failure following hemorrhage is a consequence of inflammation and cellular damage that can even be potentiated by conventional resuscitation fluids, such as RL. While resuscitating with hypertonic saline (HTS) is superior to RL, as it can mitigate some of the inflammatory events that follow hemorrhage, resuscitation with HTS is not always fully protective against inflammation and organ injury. To achieve a more complete protection, we proposed use of adenosine receptor agonists, which are potent anti-inflammatory agents, in conjunction with either RL or HTS, as supplements to prevent organ injury and cellular dysfunction following hemorrhagic shock.

Body

We began our experiments by testing the efficacy of CGS21680, a selective A_{2A} receptor agonist in preventing trauma/hemorrhagic shock (T/HS)-induced lung injury, because lung injury is the most frequent cause of death following T/HS. In our T/HS model, following anesthesia the rats received a midline laparotomy (trauma) of 3 cm. Thirty minutes after the laparotomy, T/HS rats had their blood removed to a mean arterial pressure (MAP) of 35-40 mm Hg and maintained at this level for 90 minutes by the withdrawal or reinfusion of shed blood. At the end of the shock period, the rats were resuscitated with RL at 3 times the volume of shed blood and CGS21680 was mixed into the RL to achieve a final cumulative dose of 0.5 mg/kg or 0.1 mg/kg. In contrast to our expectation, CGS21680 failed to prevent lung injury, and at 0.5 mg/kg, it even augmented it (Figure 1).

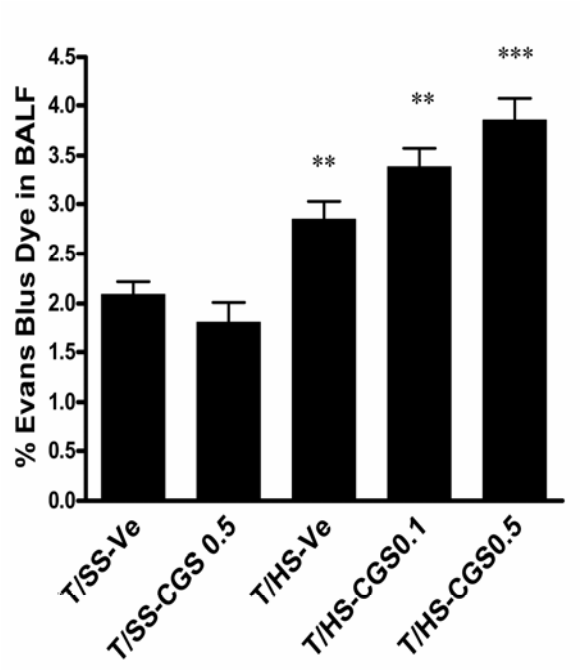

Figure 1. Treatment with CGS21680 (CGS) (0.1 and 0.5 mg/kg) fails to prevent T/HS-induced lung injury in Sprague-Dawley rats. Lung injury was evaluated by measuring permeability to Evans blue dye. Briefly, 3 hours after the end of the 90-minute shock period, the rats were injected with 10 mg of Evans blue dye through an internal jugular catheter. After 5 minutes, to allow for complete circulation of the dye, a blood sample (1.5 ml) was withdrawn from a femoral artery catheter and the plasma used to determine the plasma Evans blue dye concentration. Twenty minutes after injection of the dye, the rats were killed and the lungs harvested. Bronchoalveolar lavage (BALF) was performed on the excised lungs by lavaging the lungs 3 times with 5 ml aliquots of physiological saline. The recovered BALF was then centrifuged at 1500 x *g* at 4°C for 20 minutes to remove any cells. The supernatant fluid was then assayed spectrophotometrically at 620 nm to measure the concentration of the Evans blue dye in the BALF. The concentration of Evans blue dye in the BALF was then expressed as the percentage of that present in the plasma. Bars represent mean ± SEM of data from n = 8-15 rats per group. $^{**}p < 0.01$ and ***p < 0.005 vs. trauma/sham shock (T/SS) vehicle (ve) group.

We then assessed the effect of CGS21680 (0.5 mg/kg) on markers of liver (alanine aminotransferase, ALT), kidney (blood urea nitrogen, BUN), and muscle (creatinine kinase, CK) injury. CGS21680 (0.5 mg/kg) failed to protect against organ injury (Figure 2). Finally, we also failed to find any differences in lung NF-κB activation (as measured by detecting degradation of the negative regulator IκB) or myeloperoxidase activity (a measure of neutrophil infiltration) among the various groups (data not shown), indicating a lack of efficacy in CGS21680 in preventing lung inflammation.

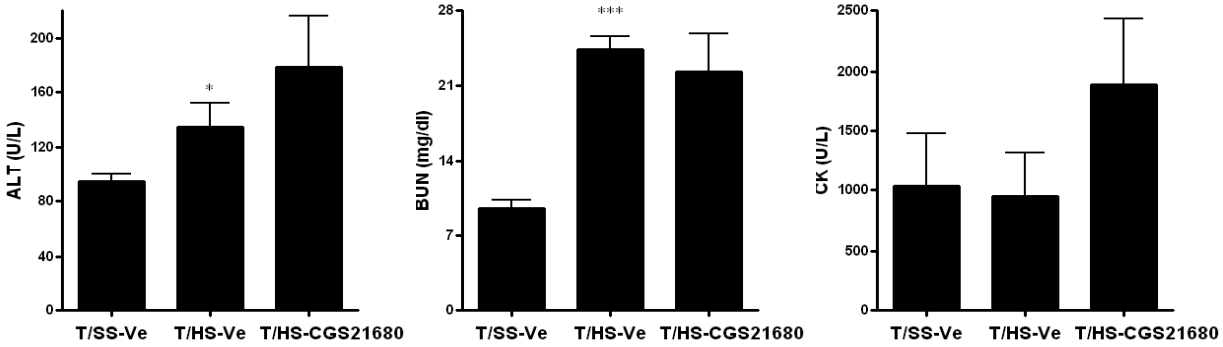

Figure 2. Treatment with CGS21680 (CGS) (0.5 mg/kg) fails to prevent liver, kidney and muscle injury in rats subjected to T/HS. ALT, BUN and CK activity were measured in plasma samples taken 3 h after the end of the 90-min shock period using a clinical chemistry analyzer system (VetTest8008, IDEXX Laboratories). Data are the mean ± SEM of n = 8-15 rats per group. $^{**}p < 0.05$; $^{***}p<0.005$.

We also monitored blood pressure and found that CGS21680 caused a severe, long-lasting decrease in blood pressure following resuscitation in both T/HS and trauma/sham shock (T/SS, control) rats, which failed to recover until the end of the experiment (data not shown). We believe this severe decrease in blood pressure can explain why in our current experiments in which CGS21680 was injected slowly with the resuscitation fluid (for up to an hour), organ injury was potentiated. This is in contrast to our previous study showing a lung protective effect of CGS21680 (1), in which CGS21680, given as bolus, caused a much less substantial and shorter lasting blood pressure. Based on our current results with CGS21680 mixed into the resuscitation fluid, we concluded that such an approach fails to provide benefit in hemorrhage, and further mechanistic studies with CGS21680 were not conducted.

We then continued our studies by evaluating the potential of the selective A_{2B} receptor agonist BAY 60-6583 to prevent lung injury. Our results indicate that BAY 60-6583 (0.5 mg/kg) mixed into RL provided an almost complete protection against increased lung permeability (Figure 3). In addition, administration of BAY 60-6583 did not have any adverse effects on the MAP in either T/HS or T/SS rats (data not shown).

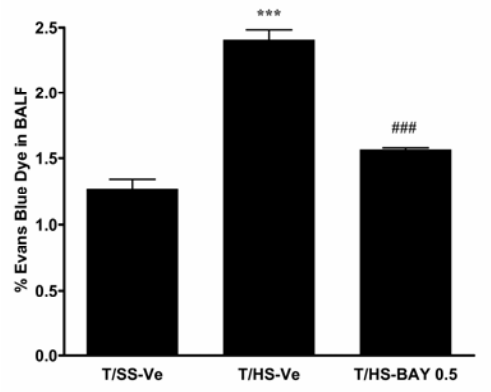

Figure 3. Treatment with BAY 60-6583 (BAY) (0.5 mg/kg) prevents T/HS-induced lung injury in Sprague-Dawley rats. Data are mean ± SEM of n = 5-6 rats per group $^{***}p<0.005$ vs. T/SS-Ve; ###p<0.005 vs. T/HS-Ve. (Ve-vehicle).

Thus, in the next funding period, we will study in detail the mechanisms whereby A_{2B} receptor activation protects against lung injury in rats subjected to T/HS.

Key research accomplishments

- **The A_{2A} receptor agonist CGS21680 fails to protect against T/HS-induced lung, liver and kidney injury, and causes severe hypotension when administered over a prolonged period mixed into the resuscitation fluid.**
- **The A_{2B} receptor agonist BAY 60-6583 prevents T/HS-induced lung injury without any adverse cardiovascular effects.**

Reportable outcomes

Dr. Gyorgy Hasko (3 month effort) led the studies. Alexey Trepakov, MD was hired as a full time (12 month effort) postdoctoral fellow to conduct most of the animal surgery studies. Balazs Csoka, PhD (12 month effort) and Balazs Koscso, BS (12 month effort) were hired to perform some of the animal surgeries and to conduct most of the biochemical assays.

Conclusion

Activation of A_{2B} but not A_{2A} receptors is a promising approach for the prevention of hemorrhage-induced lung injury. A_{2B} receptor agonists should be developed for human use to treat trauma-induced organ failure.

References

1. Hasko, G., Xu, D. Z., Lu, Q., Nemeth, Z. H., Jabush, J., Berezina, T. L., Zaets, S. B., Csoka, B., and Deitch, E. A. (2006) Adenosine A2A receptor activation reduces lung injury in trauma/hemorrhagic shock. *Crit Care Med* **34**, 1119-1125

Appendices

N/A